5 IMMUNOASSAY

5.1 Objectives

5.1.1 Understand and explain immunoassay.

5.1.2 Understand the theory of commonly used immunoassay testing methods.

5.1.3 Understand the theory and practice of fluorescence polarization immunoassay (FPIA), and specifics of operation of the Abbott Axsym or TD_x™ instrument.

5.1.4 Prepare tissue specimens for analysis by Axsym or TD_x.

5.1.5 Operate the Abbott Axsym or TD_x™ instrument as per toxicology technical procedures manual.

5.1.6 Interpret results by thoroughly explaining the instrument printout.

5.1.7 Understand the quality control aspects of Axsym or TD_x testing.

5.2 Estimated Time: Four weeks (as ½ days)

5.3 Methods of Instruction

5.3.1 Lectures

5.3.1.1 Principles of immunoassay

5.3.1.2 Types of immunoassays

5.3.1.3 Components and operation of Axsym or TD_x

5.3.1.4 Specimen preparation

5.3.1.5 Specimen analysis

5.3.1.6 Result interpretation

5.3.2 Literature Review

5.3.2.1 Goldberger, B. & Jenkins A. 1992. *Testing of Abused Drugs in Urine by Immunological Techniques.* In Service Training, AACC.

5.3.2.2 Toxicology Technical Procedures Manual

5.3.2.3 Axsym or TD_x Operator Manual, Abbott Laboratories Diagnostic Division.

5.3.2.4 Axsym or TD_x System Continuing Education Seminar; Abbott Laboratories, Abbott Customer Commitment Network

5.3.2.5 Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 301-312.

5.3.3 Demonstration

5.3.3.1 FPIA analysis will be observed from beginning to end and notes will be taken by the Trainee.

5.3.4 Laboratory Exercises

5.3.4.1 Analyze one batch of 15 blood specimens by FPIA screening for at least 2 drugs of abuse. At least 5 of the specimens will be above the cutoff concentration and at least one specimen below the cutoff.

5.3.4.2 Perform analyses for at least 2 therapeutic drugs by using blank blood and one specimen known to be positive for acetaminophen, carbamazepine, phenytoin, salicylate or valproic acid.

5.3.4.3 Calibrate one assay.

5.3.4.4 Perform all daily, weekly and monthly routine maintenance.

5.4 Evaluation

5.4.1 Written Examination

5.4.1.1 This will be administered as a "take home" exam.

5.4.2 Laboratory Competency Testing

5.4.2.1 Qualitative – a series of at least 10 previously analyzed blood specimens will be presented to the Trainee for a routine DUID panel according to the Toxicology Technical Procedures Manual. Qualitative results obtained by the Trainee must agree with previous results.

5.4.2.2 Quantitative – 5 previously analyzed blood specimens will be presented to the Trainee for quantitation of both salicylate and acetaminophen. Results obtained by the Trainee must agree within 20% of the previous results per the Toxicology Technical Procedures Manual.

5.4.3 Courtroom Exercise

5.4.3.1 The Trainee must be capable of answering questions on this Module such as would be expected in a courtroom scenario.

5.5 Examination Questions

5.5.1 Explain the advantages and disadvantages of screening for the presence of drugs.

5.5.2 Describe the following three different types of immunoassay: radioimmunoassay (RIA), enzyme immunoassay (EIA), and fluorescence polarization immunoassay (FPIA).

5.5.3 Explain the following terms as they apply to FPIA: antigen, antibody, monoclonal/polyclonal antibody, fluorescence polarization, cross-reactivity, cutoff, true-positive, false-positive, sensitivity, false negative and specificity.

5.5.4 Distinguish between homogeneous (e.g. enzyme multiplied immunoassay technique (EMIT)), and heterogeneous immunoassays (e.g. enzyme linked immunosorbent assay (ELISA)).

5.5.5 Explain cross-reactivity stating advantages and disadvantages. Include the significance of immunoassay specificity for a specific drug vs. the specificity for a drug class.

5.5.6 Name the chemical compound that is the primary target of the antibody in each of the FPIA assays.

5.5.7 Explain the effect of the size and rotation rate of the tracer on polarization.

5.5.8 Explain the relationship between net polarization and the concentration of the drug being determined.

5.5.9 Describe the components of the Axsym or TD_x kits and explain the purpose of each. Why are urine Axsym or TD_x kits used for blood specimens?

5.5.10 Explain the reasons for sample preparation (tissue dilution and homogenation , protein precipitation) prior to analysis.

5.5.11 Describe the components of the Axsym or TD_x instrument and explain the purpose(s) of each.

♦ **End**